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GENERAL CONSIDERATION OF PROBLEMS IN SHRIMP NUTRITION[1]

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ABSTRACT

There are four problems which face the shrimp nutritionist. These are (1) selection of optimal experimental environment, (2) the inherent variability among shrimp, (3) the selection of the form of food and its ingredients, and (4) the presentation of the data in a form useful both to other investigators and to the commercial mariculturist. Data illustrative of each of these problems are presented, and a request is made for the presentation of certain minimal information in each shrimp nutritional study.

INTRODUCTION

Food preferences and nutrition of penaeid shrimp are of prime concern. This was one of the conclusions reached at the World Scientific Conference on the Biology and Culture of Shrimps

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and Prawns in 1967. Since that time, various groups, stimulated by the interest of industry in the possibility of farming shrimp commercially, have begun studies directed primarily toward the development of a diet useful to shrimp farmers.

Several studies have been conducted in the United States to develop a food which would produce satisfactory growth of penaeid shrimp native to the Gulf of Mexico in experimental tanks (Subrahmanyam and Oppenheimer, 1970 and 1971; Sick et al., 1972; Venkataramaiah et al., 1972; Meyers and Zein-Eldin, 1972; and Grajcer and Neal, 1972). Others have studied feeding in ponds: Broom (1971) and Latapie et al. (1972) in Louisiana; Cailloüet et al. (1972), and Tabb et al. (1969) in Florida; and Elam (1972) in Texas. Unfortunately, the composition of the diets, the sizes of the animals used, the species, and even the experimental and environmental conditions, including the presence of natural foods, have been so different that few comparisons among these studies are possible.

It is the purpose of this paper to discuss some of the problems facing the experimental nutritionist and illustrate them with data from our own experiments. The problems are of fourfold: (1) selection of an optimal experimental environment, (2) the inherent variability in the animals, (3) the selection of the form of food and its ingredients, and (4) the presentation of the data in a form useful to other investigations and to the commercial mariculturist.

Comparisons to be made in this paper will be limited to studies which have been semicontrolled, and we will omit pond studies. We have assumed that economically successful shrimp culture in the United States is possible only on an intensive basis in controlled systems, necessitating high stocking rates, and feeding of a prepared, formulated diet.

SELECTION OF OPTIMUM EXPERIMENTAL ENVIRONMENT

The choice of environmental conditions that are standard, reproducible, and optimal is the first problem of the nutritionist. These problems are relatively simple in terrestrial animals which can be adequately housed in an environment of known temperature, humidity, and oxygen. Aquatic animals, however, pose an additional problem for the biologist. We must have knowledge of temperature and salinity requirements, and some indication of the effects of possible contaminants in the water, as well as providing adequate aeration of the water and effective removal of wastes.

Our experimental conditions were established based on a background of earlier work in defining environmental conditions of temperature and salinity in which postlarval and juvenile penaeids survive and grow (Zein-Eldin, 1963; Zein-Eldin and Aldrich, 1965; Zein-Eldin and Griffith, 1966, 1969).

Water Exchange

We have usually conducted nutrition experiments indoors in the 60-liter glass aquaria (30.5 x 75 x 30.5 cm) containing 46 liters of seawater and fitted with Eureka[2] under-gravel filters. These aquaria have been described in detail in earlier papers concerning the effects of environmental factors on postlarval shrimp (Zein-Eldin and Aldrich, 1965; Zein-Eldin and Griffith, 1966). The filter bed consisted of oyster shell which had been washed and later dried in an oven at 177 C, topped with layers of blasting sand which was boiled for 30 minutes for sterilization. Seawater was first filtered through a 5 μ filter, and then passed through a quartz ultra-violet sterilizer. Aquaria were placed in temperature control rooms in which temperatures were maintained at 28 $\pm$ 1 C. Salinities (28 $\pm$ 2 ppt) and temperatures were measured daily. Aquaria were placed before fixtures fitted with two 40-W clear-white fluorescent bulbs, and a light cycle of 12 hours light and 12 hours dark was maintained. The size of these aquaria, however, limited the number of animals that could be fed. Attempts to replicate these small experiments in the larger tanks in our flow-through seawater system were uniformly unsuccessful.

We had thought that the flow-through system would offer several advantages. In addition to offering comparability with other workers (Subrahmanyam and Oppenheimer, 1970, 1971; Sick et al. 1972), the continuous flow of seawater (maintained at constant temperature by a heat exchanger) would wash away uneaten food particles and dilute or eliminate waste products.

To test this system, we compared growth of 100 brown shrimp (Penaeus aztecus) in each of two fiberglass tanks (61 x 152 x 92 cm) containing a sand substrate. One tank had a beneath-the-surface filter of the type described above. Water was recirculated within the tank; warmed water flowed through the other tank directly from our circulating seawater system, and was neither filtered nor sterilized. Although temperatures were maintained as close as possible to 28 C and salinities at about 28 ppt, some fluctuation did occur. A light-dark cycle was maintained, but the quantity of light reaching individual tanks was considerably less than that present in the controlled situation.

Shrimp used were hatchery-reared postlarvae that had been fed live brine shrimp for 1 month after reaching the postlarval stage. Our control diet, which was previously described by Meyers and Zein-Eldin (1972) and is described in Table 1, was fed to these animals on an ad lib. basis, that is, the ration fed was increased or decreased daily depending upon the amount the animals ate. Feeding rates varied with the size of the animals,

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[2]The use of trade names in this publication does not imply endorsement of commercial products.

being as high as 100% of the body weight per day in early stages and decreasing to about 10% with the largest animals.

The mean weights of shrimp sampled from the two tanks are presented in Figure 1A. After 17 days juvenile brown shrimp in the flow-through system were apparently larger than those in the recirculated system. By the end of 24 days, however, the animals in the recirculated system were significantly larger, and were approximately twice the weight of the animals in the flow-through system after 58 days. Biomass differences were more convincing--a final biomass of 95 g was present in the recirculated tank vs. only 17.5 g in the flow-through system. Since 25 animals were removed from each tank each time a measurement was made, the decline in numbers was not due entirely to mortality.

These results, suggesting the superiority of a static water system, were further tested in a second experiment in which we added an intermediate condition, i.e., a recirculated system in which half the total volume of water was replaced weekly. The superiority of the recirculating systems is demonstrated in Figure 1B. Shrimp in each of the recirculated tanks were almost double the weight of those in the flow-through tanks after 56 days. At that time biomass in the recirculating tank was ten times that in the flow-through tank, and the number surviving was about five times that in the flow-through system. During this experiment, 10 animals were removed from each tank for each growth measurement.

There seems to be little or no advantage in replacing water weekly; all subsequent experiments have been conducted in recirculating systems with bottom filters. It would appear that the organic-rich water of the recirculating system is more successful in promoting the growth of juvenile brown shrimp than the cleaner flowing water.

Stocking Density

A second environmental parameter, that has not been uniform in feeding experiments conducted by different groups, is the stocking rate of the experimental animals. A decision regarding the appropriate density involves the effect of crowding on growth in a particular system, the need for large numbers of animals so that real differences can be recognized, and the type of grow-out system in which experimental findings will be applied to commercial production.

In the laboratory nutritional experiments in 60-liter aquaria, we usually hold 10 to 15 animals. Initial weight of individual shrimp has varied from 0.5 to 1.5 g, with initial total biomass ranging between 5 and 15 g per tank (20 g/m^2 to 60 g/m^2). Under such conditions (no natural food, semisterile environment, artificial light source), the final biomass has risen from 43 g/m^2 to 172 g/m^2 of brown shrimp fed our standard

diet. These values are considerably higher than those reported by others (Subrahmanyam and Oppenheimer, 1970; Sick et al., 1972; Venkataramaiah et al., 1972).

For most economic applications in shrimp culture, however, we feel that even these densities are far too low. We have, therefore, conducted two experiments with shrimp held in indoor fiberglass tanks described previously. Initial stocking rates for the brown shrimp were 100, 200, 400, and 800 animals per tank (108, 216, 431, and 863/m^2) at an initial total length (tip of rostrum to tip of telson) of 15 mm and weight of 23 mg. Within 1 month a disease occurred in shrimp inflicting heavy mortalities in some tanks. The animals which survived this disease, identified as a Vibrio alginolyticus infection by Dr. Donald Lightner of our pathology department, were all from tanks stocked with 400 or 800 animals. These survivors (F in Figure 2) grew fairly well during the succeeeding 12 weeks, and in one tank they attained a maximum biomass of 233 g/m^2. After 16 weeks in this tank, which was stocked with 400 shrimp, only 64 of the 280 not removed for measurement survived.

In a second experiment with white shrimp (Penaeus setiferus) only two densities, 400 and 800 (430 or 860/m^2), were tested. Juveniles initially were 30.8 $\pm$ 2.4 mm total length and 0.18 $\pm$ .04 g total weight (Table 2). As in other experiments, animals were fed ad lib. Initial biomasses were 73 g and 145 g/tank (78 g/m^2 and 156 g/m^2). The animals removed biweekly for weight and length measurements were returned to the tank.

As indicated in Figure 3, survival, expressed either as percentage of initial stocking or as actual numbers of animals, was less in the more densely stocked tanks after the 20th day. Although some of the deaths were undoubtedly due to the stress during handling (this species seems to be considerably more delicate than the browns) it also seems evident that there were mortalities due to the density of animals.

Various authors using other experimental systems (Sick et al., 1972; and Subrahmanyam and Oppenheimer, 1970) stated that 40-80 g/m^2 is an optimum stocking density. In our tests at only four sampling periods were biomasses this low; 17 of 32 were between 100 and 200 g/m^2, and 10 were greater than 200 g/m^2. The maximum (and final) biomass of 368 g/m^2 consisted of 123 shrimp at a density of 132 animals/m^2.

Although the statistical difference in final weight between the two stocking rates was significant (pooling all individual measurements from the two groups), selection of the lower stocking density as preferable would be made not on the basis of final size, but rather on the increased survival rate at the lower density. This experiment is also not definitive in choice of stocking level, since the presence of additional light, modification of filter, and even a change in water volume might well affect the possible stocking intensity. Mock et al. (in press)

have reported good growth at biomasses up to 690 g/m^2 in another type of recirculating tank.

VARIABILITY IN ANIMALS

It is impossible for an investigator to make intelligent comparisons between groups of animals without an adequate knowledge of the normal variability within the experimental population. Differences in growth rates among individual shrimp seem to be greater than those observed in some other animals. This applies to animals derived from nature (see size ranges reported by Zein-Eldin and Aldrich (1965) for postlarval brown shrimp held 30 days) and to those animals hatched in the laboratory. This inherent variability makes interpretation of data even more difficult when comparisons are made of groups of only 10-15 animals; a mean may be markedly affected by the presence of one rapid or one slow growing shrimp. Although we attempt to compensate by choosing animals of a uniform size with a known feeding history, variability is still a factor which must be recognized by each investigator.

Within Experimental Groups

Datum from the experiment above is a good example of the variability inherent in both the experimental systems and the shrimp themselves. The brown shrimp and white shrimp used in those studies were hatched at the Galveston Laboratory. The history of the experimental animals, including their foods and environmental conditions used during culture, was thus known. Animals were transferred to our section as postlarvae of 4-5 mm total length. Postlarvae were fed live brine shrimp and were measured regularly (weight and total length) for approximately 1 month before beginning nutritional studies. At the final measuring period each animal was weighed to the nearest mg.

Comparison of the mean final size using Student's t Test for all observations (Table 2) showed that there were differences significant ($P < .01$) between mean lengths of shrimp in the two tanks originally stocked with 400 shrimp per tank. There were significant differences ($P < .01$) between shrimp in the two tanks originally stocked at 800 per tank. These differences between replicates are as great or greater than those used by others to select between diets or ingredients in diets.

Between Experiments

Figure 2 presents data taken from some of our experiments illustrating the growth differences we have observed between similar experiments. All of these were conducted in 60-liter aquaria as described above, and all animals were fed the standard food ad lib. With this food the size of 0.5 g shrimp has

approached and sometimes exceeded 3 g in 50 days. The lowest curve represents a large tank (61 x 152 x 61 cm) stocked initially with 430 animals/m^2 and subjected to a Vibrio infection. Following recovery from the infection, the animals responded fairly well, and were maintained in the laboratory for almost 6 months.

The variations in growth shown here are in part attributable to differences between populations of shrimp. These data together with those of the replicate large tanks presented earlier suggest that small differences between groups fed different diets must be interpreted cautiously.

SELECTION OF FOOD

Another problem consists of the type of food to be presented to the animals. Not only must a food retain its form and consistency for a number of hours in water (Meyers and Zein-Eldin, 1972), but it must be attractive and available to the stage of animal to which it is presented. Because shrimp seem to find most of their food by touch, the food form, whether it is a flake in the water column or a pellet on the bottom, should be determined by the behavior of a given stage. The tendency of small postlarval penaeids to spend much of their time in the water column may explain the success of live brine shrimp as a food. Since brine shrimp swim actively in the water column, they are frequently encountered and thus ingested.

Mock et al. (in press) repeatedly fed a commercial flake food (TetraMarin®) to postlarval shrimp. In a recent experiment we have fed this food as well as an experimental flake to juvenile brown shrimp; other diets fed in this experiment included the extruded standard supplemented with vitamins and cholesterol and another extruded food low in total and animal protein (Figure 4).

Shrimp survival was lowest with the two flake foods, perhaps because of the enrichment of the water with organics derived from decomposing feed which promoted undesirable bacterial growth. In addition, shrimp fed TetraMarin® developed a growth of colorless stalked algae, covering the appendages (including the eyestalks) until the animals appeared hairy or fuzzy. This growth was shed as the animals molted.

Animals fed the low protein extruded diet (Figure 4) were somewhat smaller, but 100% survived. Molting frequency was reduced in this group during the first 2 weeks of the experiment and then approached that of the standard group. This suggests that adaptive enzymes may be formed either by the shrimp themselves or by bacteria present within the gut.

Microbiological aspects of the shrimp digestive tract must be considered for a total picture of the animal's nutrition. Hood, et al (1971) noted high bacterial concentrations (10^6 to 10^7 cells/g) within the digestive tract of white shrimp, although

a relatively limited diversity of morphological and physiological types was observed compared with bacteria isolated from the environment of the animal. Bacteria from the shrimp gut exhibited rapid assimilation of carbohydrates (ca. 4 hours), whereas predominant sediment and seawater organisms utilized fewer carbohydrates and required 48-72 hours for assimilation. Gut bacteria were predominantly chitinoclastic, with many producing proteinase, lipase, and amylase, often within 24 hours. The shrimp proventriculus contained a larger diversity of physiological types than found in the gut. Evidence suggested that the microbial complement of the digestive tract was determined by the biological and biochemical characteristics of the ingested food particles as well as by the ability of certain bacteria to resist digestive processes, i.e., low pH, enzymatic activity, and mechanical action of the proventriculus.

PRESENTATION OF DATA

The final problem confronting us is the selection of a method of expressing data so that comparisons may be made between diets, as well as experimental conditions, proposed by various investigators. Because our data must ultimately be of use to the commercial mariculturist, the use of a simple, direct presentation of data is most desirable.

Growth has frequently been presented as percent increase, either in mean weight or biomass. The interpretation of this value, usually defined as:

$$\frac{\text{Final Weight - Initial Weight}}{\text{Initial Weight}}$$

(Kitabayashi et al., 1971) is complicated when comparing groups of animals of differing initial weights, for percent increase is inversely related to initial weight. Furthermore, the use of such a value magnifies differences among groups and when only an average is used, ignores variability between and among groups. Thus, calculation of the mean percent increase for the stocking-rate experiment cited above would yield percent increases of 1,040 and 1,450 for the replicate tanks stocked at 400 shrimp per tank. Use of this value for the selection of dietary ingredients may be justified in animals for which we have considerable evidence of replicable growth rates (Wistar strain rats, certain strains of hogs and cattle, and chickens) but as yet we have no such data bank for shrimp.

Since growth is a function of time, we should like to propose the use of a simple method of data presentation, i.e., mean weight (or biomass) vs. elapsed time. In Figure 5 the growth of various species of Penaeus fed known diets, both natural and formulated is compared. These lines represent the fastest growth reported by a given investigator. All data have been taken from studies in semi-intensive systems rather than ponds. Some data

have been recalculated from data presented as percent increase. Other data have been omitted because there was insufficient information (lack of initial or final size; numbers of animals tested, etc.) to permit the calculation of a line.

The best growth rates shown here are for P. aztecus fed natural food, either mussel (Beard, 1971) or shrimp heads (Grajcer and Neal, 1972). In those studies, 0.5 g shrimp reached a weight of at least 3 g after 50 days. Better growth with natural foods has been reported with some oriental species by Beard (1972) and with the white shrimp by J. R. M. Forster (personal communication).

Presentation in this manner permits us to compare growth of brown shrimp fed natural foods with the growth we report here for shrimp fed the standard extruded diet (Figure 4). It is evident that brown shrimp grow as well, that is from 0.5 g to 3 g in 50 days, when fed the extruded diet as when fed natural foods. More data which can be compared to those are needed.

CONCLUSION

We have discussed some of the problems facing the experimental nutritionist. There are yet others which concern the practitioner of shrimp mariculture. An economical food must produce a dependable amount of growth at a reasonable cost. At present we are not able to standardize the growth of the cultured animal, but we can standardize the components of the diet.

Careful consideration must be given to designation of the origin of particular components of the diet. For example, shrimp meal, a major constituent of our diets, varies greatly even in proximate analysis between variously processed samples (Table 3) adapted from Meyers and Rutledge (in press). Greater differences occur between meal samples from regions where different species of shrimp are processed. These dissimilarities become significant when protein levels of the diet must be standardized to evaluate the effect of a diet on growth. Minor differences between the same product are normal, but careful designation of the origin of the material will allow more accurate comparison of data from different investigators.

In conclusion, we would ask for the following information as the minimum necessary data in publications evaluating experimental diets for shrimp: (1) A description of the experimental animals, their species, source, and history; (2) a description of environmental conditions including tanks, water systems, density of animals, and temperatures; (3) a description of feeds used, their ingredients, and the method of preparation; and (4) data concerning the growth of shrimp on each diet, preferably in the form of sizes of the animals vs. time.

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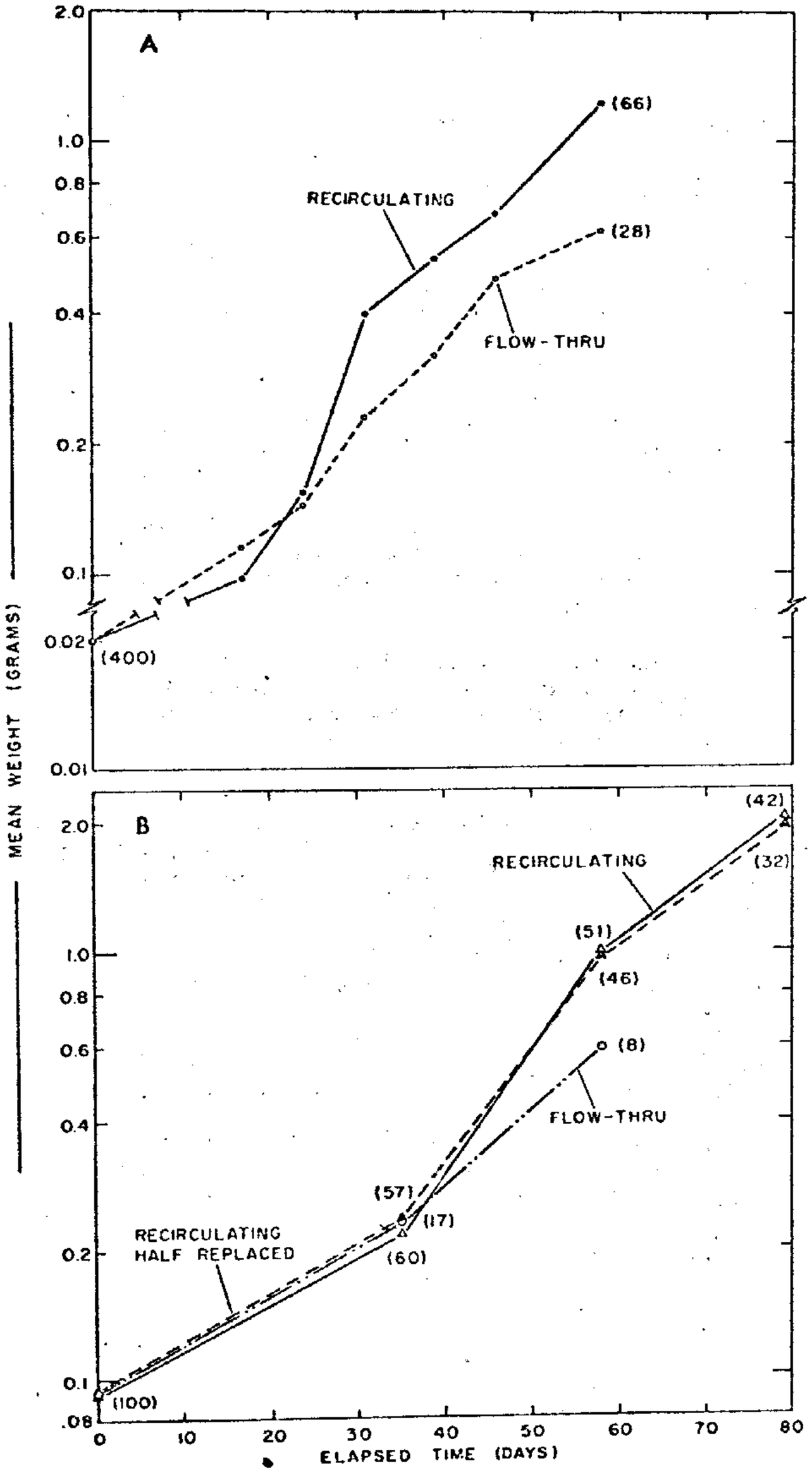

Figure 1. Growth of P. aztecus in recirculating vs. flow-through system, numbers surviving in parentheses.

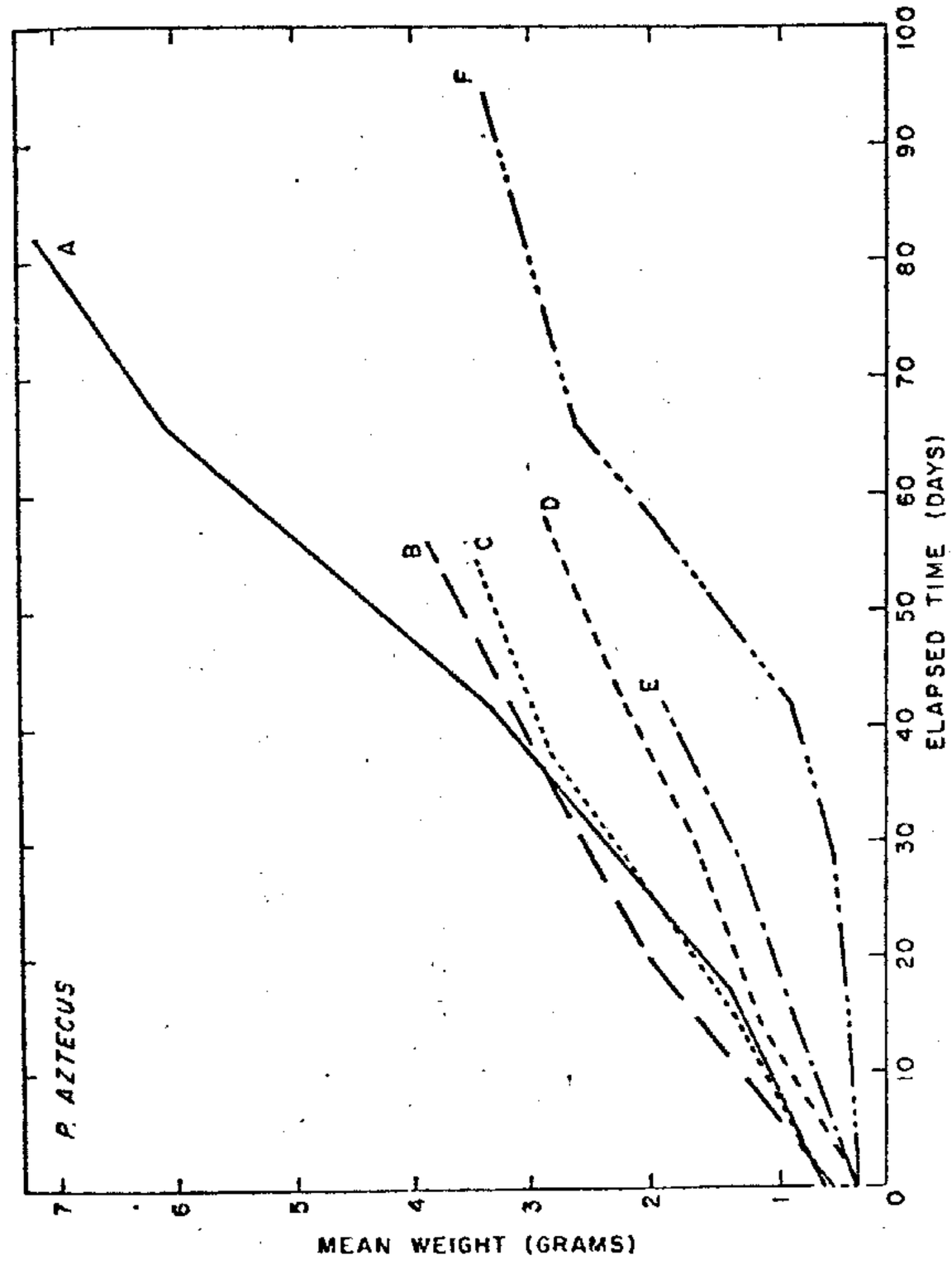

Figure 2. Variability in growth between groups of P. aztecus fed the standard diet. Groups A through E were tested at different times and held in the 60-liter glass aquaria. Group F was held in the 61 x 152 x 92 cm fiberglass tanks (0.93 m^2).

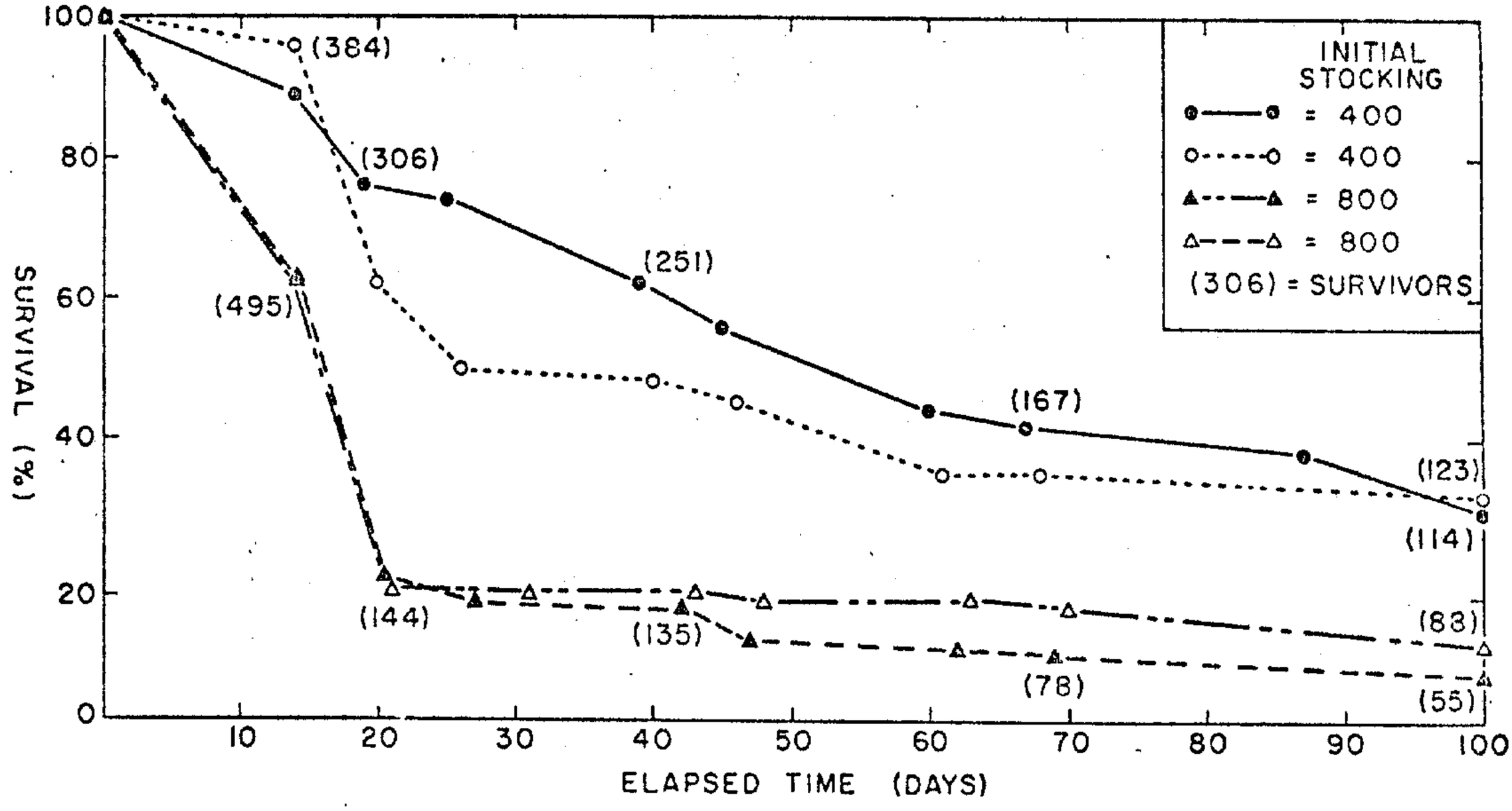

Figure 3. Survival of white shrimp stocked in 61 x 152 x 92 cm tanks (0.93 m^2) at levels of 400 and 800 per tank.

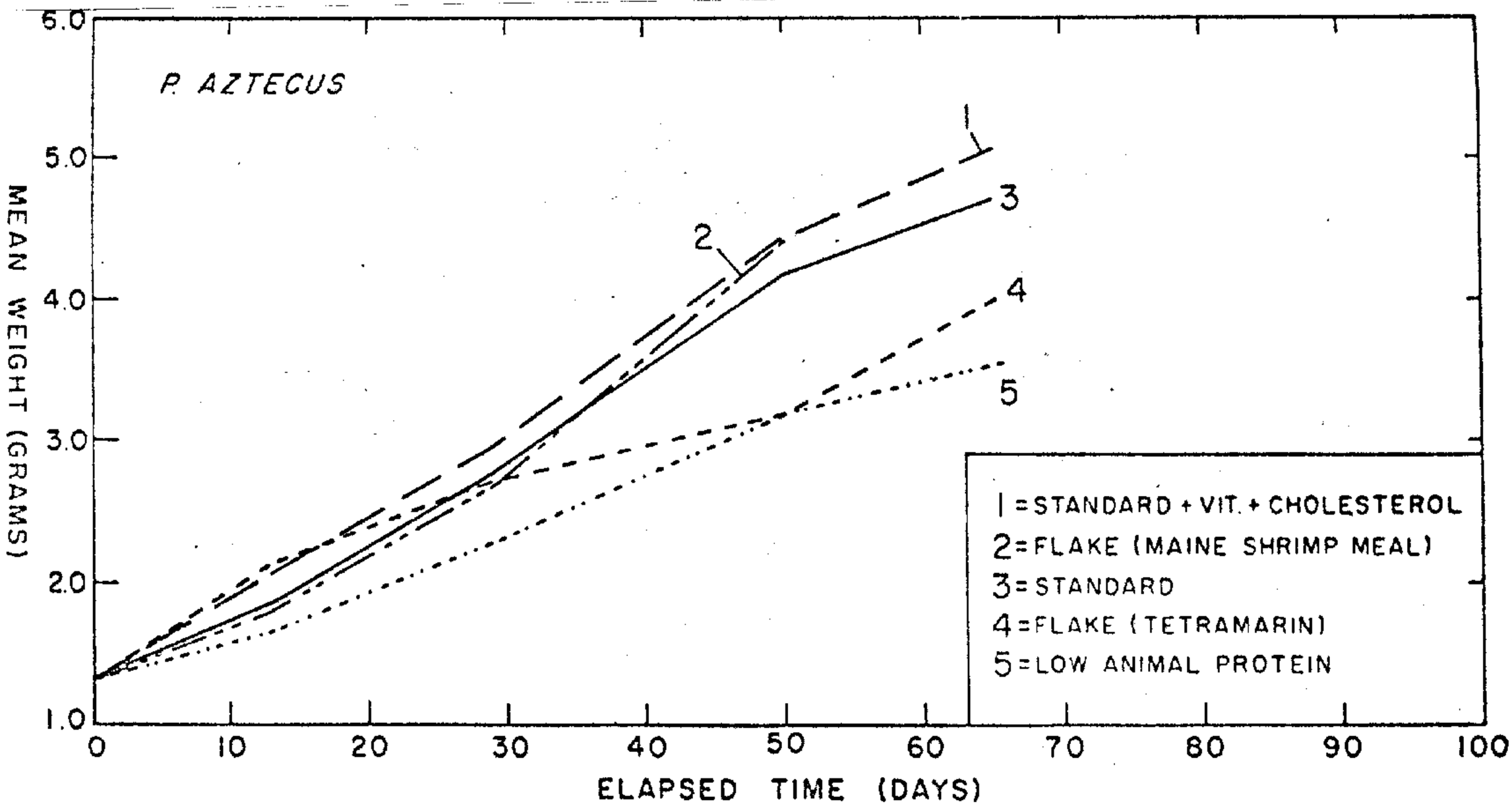

Figure 4. Growth of P. aztecus in 60-liter glass aquaria, fed several test diets.

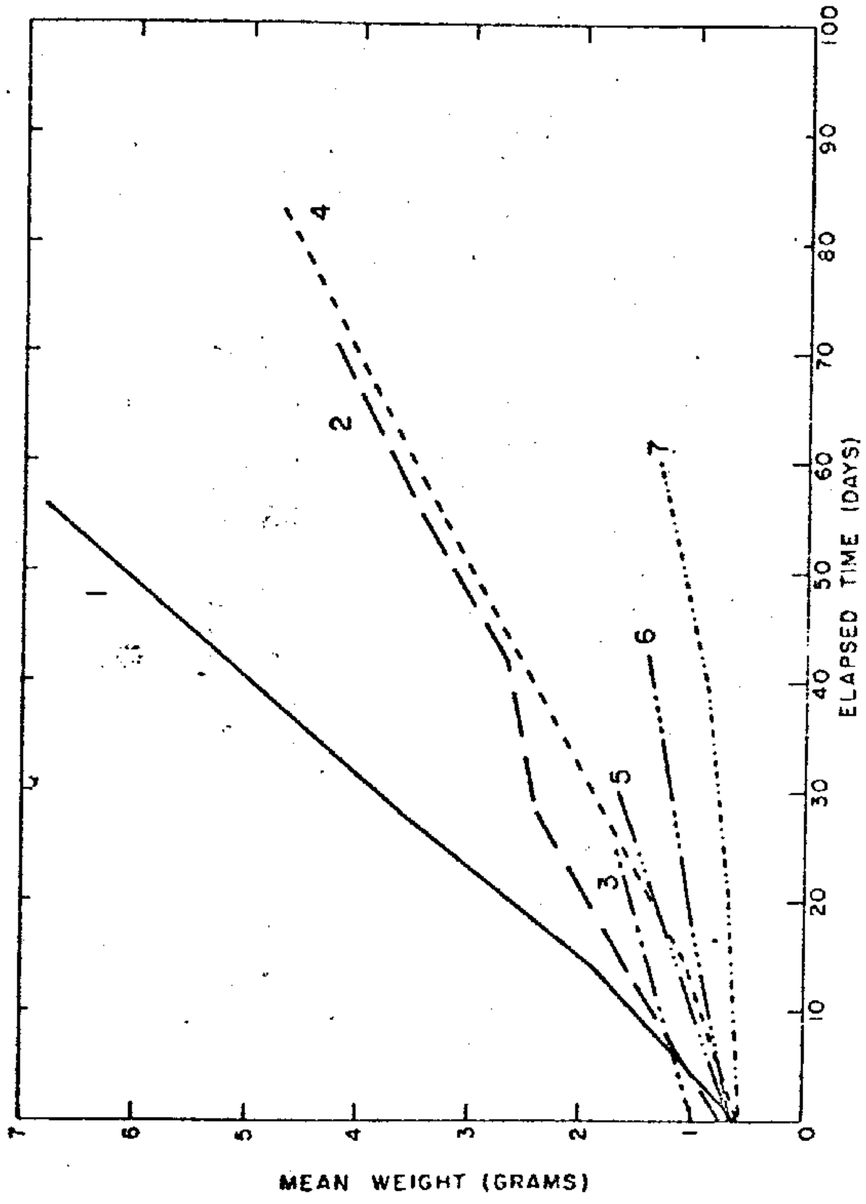

Figure 5. Growth of various penaeid species held in semicontrolled systems: (1) P. aztecus - 25/m²-Beard (1972); (2) P. aztecus - Grajcer and Neal (1973); (3) Penaeus sp. - Lee and Liao (1970); (4) P. aztecus - 166/m² - Beard (1972); (5) P. japonicus - Kitabayashi et al. (1971); (6) P. aztecus and P. duorarum - Subrahmanyam and Oppenheimer (1970); (7) P. monodon - Lee (1971).

Table 1. - Percentage composition of standard shrimp diet

Component	Percentage
Shrimp meal, sun-dried[1]	31.5
Fish meal (menhaden)	8.0
Soybean meal[2]	3.0
Rice bran[3]	49.0
Vitamin mix[4]	2.0
Fish solubles	2.0
Lecithin[5]	1.0
Kelgin[6]	2.5
Sodium hexametaphosphate[7]	1.0

[1]Blum and Bergeron, Houma, Louisiana.

[2]Promine-D, Central Soya Company, Chicago, Illinois.

[3]Protex-20, Riviana Foods Inc., Houston, Texas.

[4]Nutritional Biochemicals, Cleveland, Ohio.

[5]Alolec (Soybean Lecithin), American Lecithin Company, Long Island, New York.

[6]High velocity (HV) algin, Kelco Company, San Diego, California.

[7]Calgon.

Table 2. - Growth of white shrimp stocked in 61 x 152 x 92 cm tanks (0.93 m^2) at levels of 400 and 800 per tank

Elapsed time (weeks)	No.	Biomass (g)	Mean weight ± standard deviation (g)	No.	Biomass (g)	Mean weight ± standard deviation (g)
	----------Tank 1----------			----------Tank 2----------		
0	400	73	0.18 ± 0.04	400	73	0.18 ± 0.04
2	358	106	0.30 ± 0.05	384	119	0.31 ± 0.11
4	305	108	0.30 ± 0.09	200	86	0.43 ± 0.17
6	251	167	0.67 ± 0.17	188	152	0.81 ± 0.21
8	200	226	1.13 ± 0.28	160	209	1.30 ± 0.30
10	167	238	1.43 ± 0.32	147	254	1.73 ± 0.47
12	152	290	1.91 ± 0.71	140	315	2.25 ± 0.38
15*	114	231	2.05 ± 0.50	123	343	2.79 ± 0.57
	----------Tank 3----------			----------Tank 4----------		
0	800	145	0.18 ± 0.04	800	145	0.18 ± 0.04
2	507	152	0.30 ± 0.09	495	134	0.27 ± 0.07
4	135	72	0.53 ± 0.21	148	55	0.37 ± 0.12
6	135	107	0.79 ± 0.29	148	107	0.72 ± 0.18
8	88	114	1.29 ± 0.43	137	152	1.11 ± 0.32
10	78	149	1.91 ± 0.43	130	221	1.70 ± 0.36
12	66	125	1.90 ± 0.45	115	217	1.89 ± 0.41
15*	55	138	2.51 ± 0.48	88	178	2.03 ± 0.42

*All individuals measured at this time; all other biomass and mean weights based upon samples of 10% of the counted number in the tank. Tanks had bottom area of 0.93 m^2.

Table 3. - Proximate analyses of two Louisiana-processed shrimp meals

Shrimp meal product	Content					
	Protein[1]	Fat	Fibre	Moisture	Ash	Ca	P
	----------%					
Dehyndrated	28.5	1.3	21.4	7.5	38.2	15.0	2.2
Sun-dried	47.8	2.9	10.4	9.1	26.9	7.0	1.5

[1]Corrected for chitin.